

EXPERIMENTAL SYPHILIS RESEARCH— A REVIEW OF SOME OF THE RECENT FINDINGS*

By ERNEST K. STRATTON, M.D.
San Francisco

IN selecting a subject for a chairman's address, it would be only natural for one to speak on the phase of medicine in which he was particularly well versed. In my own case, however, I find myself practically devoid of any so qualified a subject. This, together with the fact that my paper has the mean advantage of being closed to discussion, has lead me to select a subject which has not only held considerable fascination for me, but about which I have known very little. Had I known how voluminous, and I use the word literally, I should find the literature on experimental syphilis, I would probably not have welcomed this opportunity for its review quite so wholeheartedly as I did, for, in the few moments allotted to me here, I can only touch on such highlights of my reading as seem outstandingly significant. These represent the general conclusions of those research workers in this field whose standing cannot be questioned, since beyond and about their heads there seems to be an amazing amount of controversy.

NOGUCHI'S STUDIES

As you probably know, the *Spirochaeta pallida* was first grown in pure culture by Noguchi in 1911. He was subsequently successful in obtaining positive animal inoculations from his subcultures as well as his primary cultures. His description of the media used, method of preparation, incubation, etc., reads very simple; but it is surprising to note how many investigators have been unable to cultivate this organism. Most workers are unsuccessful in their attempts to inoculate animals from the primary culture after it is forty-eight hours old and all of the subcultures, with the exception of Noguchi's, have been negative.

The *pallida* cultures isolated, studied and reported in this country have been few. In addition to the several by Noguchi, there was one by Zinsser and another by Kolmer. All of these were isolated from lesions of rabbits' testicles infected with strains of *pallida* which had been maintained in rabbits for several generations. On the other hand, the several strains reported in Europe by Reiter, Kroo, Krantz and others have all been obtained directly from human lesions, or from the primary lesion produced in the rabbit with human material.

Since all of the *pallida* cultures, with the exception of the one reported by Noguchi, have immediately become avirulent, the possibility that each or all of them might have been growths of some of the other fine types of spirochaetes, must be considered. To quote Kolmer: "It seems evident that no definite proof as to the identification of any of the alleged *pallida* cultures can be

offered until some method is found to prolong the virulence of the cultures, or until the factors are ascertained which permit of more constant results in securing the multiplication of the virulent spirochetes in culture media, even though their multiplication is accompanied by a loss of virulence."

OTHER STUDIES

Perhaps the presence of an evolutive or granular form of the *Spirochaeta pallida*, which has been described by so many investigators in all parts of the world, is responsible for this lack of success in the culturing of the organism. As N. R. Ingraham, Jr., in his critical review of the literature, has said: "The presence of granular forms in all the parasitic genera of the family Spirochaetaceae would indicate that this phenomenon is significant of something more than mere degeneration. If a minute resistant body is the cause of syphilitic infection, the changes that would be wrought in our ideas concerning the etiology, pathology, diagnosis, therapy and prognosis of this disease need scarcely be pointed out." Briefly, as Ingraham has suggested, it would explain many of today's unsolved mysteries of syphilis, such as:

(a) The long primary and secondary incubation periods.

(b) The discrepancy between the size of the lesions and the number of *Spirochaetae pallida* present.

(c) The scarcity of division forms in the most active lesions.

(d) The rapid destruction of the microorganisms by the intravenous injection of synthetic arsenicals without an immediate cure resulting.

(e) The comparatively high incidents of relapse in spite of the most vigorous treatment.

(f) The long latent periods in which hardly a manifestation of the disease is detectable, and the *Spirochaeta pallida* never.

(g) The scarcity of typical microorganisms in the lesions of late and parasyphilitic, and even, in some instances, in congenital syphilis despite the severity of these processes.

(h) The question of paternal transmission, which is still shrouded in mystery and doubted by the majority.

Perhaps, too, this granular form also explains why the *Spirochaeta pallida* is not detectable on either darkfield examination or staining of rabbits' infected lymph-gland tissue, or in the organs of animals harboring asymptomatic infections; yet why, when these tissues are emulsified and injected into rabbits' testicles, they give positive results.

But if, on the other hand, the granular form represents a mere degeneration of the *Spirochaeta pallida*, it is important to realize that it cannot then be brought forward to explain these perplexing problems with which the clinician and pathologist are so frequently confronted.

STAINING METHODS

From the growing to the staining of the *Spirochaeta pallida*, we find that its staining in fresh smears or in tissue sections is successfully accom-

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plished either by the use of the different colored aniline dyes, basic and acid, for staining and counterstaining, or by the use of silver nitrate in varying dilutions. Of the latter, there are many described methods, each bearing the name of the investigator who first reported it. They differ only in the manner in which the preliminary treatment of the tissue is carried out, and in the reduction or precipitation of the silver salt following the treatment.

Olsen has made a comparison of the methods of staining *Spirochaeta pallida* and by all of them sections of congenital material are readily stained, although Jahnke's is the most satisfactory for this tissue. He found that for the aorta, the improved Warthin-Starry method obtains the best results, and is the only one by which any considerable percentage of organisms are demonstrated; while for staining brain tissue, Dieterle's method is best. It is apparent, then, that no one stain for the demonstration of *Spirochaeta pallida* in block or single sections has reached general perfection and, for this reason, it is much better to embed the tissue and cut single sections which can be stained by the various methods, selecting the one which is best suited to the given tissue. Before leaving the discussion of staining, let me remark that Warthin perfected a modification of his staining methods for the special purpose of demonstrating certain intracellular forms (ring forms, small spirochete forms and lymphocytic granules), which, because of their connection to typical spirochetes, he regarded as representing phases of the syphilitic organism.

QUESTIONABLE STRAINS OF SPIROCHAETA PALLIDA

Now there is the problem of the questionable strains of *Spirochaeta pallida*. Levaditi and his coworkers have contended that there is a "neurotropic" strain of *Spirochaeta pallida* (associated with the nervous system) as distinct from the "dermotropic" spirochete responsible for lesions of the skin and mucous membranes. His conclusions were based upon the comparison of a strain obtained from a case of general paresis with one isolated from a chancre.

Other investigators believe that conclusive proof which would warrant the separation of types of *Spirochaeta pallida* is still lacking. All of these factors have been shown to vary exceedingly, and to be modified by such important considerations as the time of transplant, condition of the lesion, method of preparing suspension of spirochetes, mode of infection and the type, age and condition of the animal used.

It is interesting to note that a rabbit which is inoculated with the Nichols strain (originally obtained from the spinal fluid of a patient with neurosyphilis) is found, after ninety days, to be resistant to another reinfection with the Nichols strain, although it can be superinfected with the Truffi strain, for instance—a strain originally obtained by Truffi from human chancre material. Neither of these strains, however, will penetrate the rabbits' nervous system, even though introduced directly into the spinal fluid or brain tissue.

Working on his problem, Schlossberger found that a mouse, inoculated with either of these strains, acquired an asymptomatic infection; but that, by inoculating a rabbit's testicle with this mouse's brain, the rabbit developed a primary and secondary syphilis which involved its nervous system as well. Thus it was evident that the spirochetes, as a result of a sojourn in the nerve tissue of the mouse, had acquired neurotropic properties rendering them capable of penetrating into a rabbit's nervous system.

Raiziss then carried the strain through four passages, from rabbit to rabbit, by means of transplanting the brain substance of the rabbit into the testicles of other rabbits, without the loss of this neurotropic property in these spirochetes. The term "neurotropic" is used by him, not in the sense of Levaditi, who employed it to designate affinity for nerve tissue and neutrality toward cutaneous tissue, but to denote this modified power of penetration.

IMMUNITY PROBLEMS

Delving into the question of immunity, I have found it pretty well divided into two schools of thought. The first, known as the Neisserian Doctrine, has for its adherents such men as Neisser, Finger, Kolle, Prigge and Frei. This doctrine is based on the following experiments made by Neisser. He inoculated a series of apes with syphilitic virus and produced syphilitic lesions. After the lesions had healed, he inoculated them a second time with syphilitic virus and failed to produce a second attack of syphilis in most of them. He then examined the internal organs of these apparently refractory animals, and found that they were still harboring virulent syphilitic virus. He next proceeded to treat a series of syphilitic apes with various antisyphilitic drugs and then tested the behavior of some of these animals toward a second syphilitic infection. In other treated animals, he investigated the presence or absence of syphilitic virus in the internal organs. The comparison of the results in the two sets of treated animals led him to conclude that immunity in syphilis is dependent upon the presence of a coexisting syphilitic infection, and that there is no immunity without infection—that is to say, without living spirochetes somewhere in the body—and that when these spirochetes are destroyed, the immunity or resistance disappears at once. He found also that the acquisition of a second attack of syphilis was proof that the first infection had been completely eliminated. The so-called reinoculation test for the cure of syphilis is based on this theory.

The other school of thought has as its adherents Chesney, Voegtlin and Armstrong, Adachi, Mantefel, Worms, and Breinl and Wagner. It really should be known as the Chesney Doctrine on account of the tremendous amount of investigation done by Chesney along these lines.

He believes that immunity in syphilis is not dependent upon the presence of the infection, but is an attribute which, once acquired, persists thereafter, even if the infection be eliminated. He

found that the response of a syphilitic animal to a second infection with syphilitic virus depended upon several factors, notably the material used for the second inoculation and the time at which the inoculation is made. Chesney found that rabbits could be successfully reinfected with the same strain of *Spirochaeta pallida* up to an approximate forty-five to ninety days after the primary inoculation. Thereafter they developed a resistance, which was of such a nature as to permanently protect them against a reinfection with the same strain of the *Spirochaeta pallida*. He then administered curative doses of arsphenamin to these rabbits, and after waiting several months, he obtained negative results, when he transferred their lymph nodes into normal rabbit testicles, indicating that their tissues had been sterilized of the pallida infection by the arsenic medication. He then reinoculated the rabbits so treated with the same strain of *Spirochaeta pallida* and no chancre developed. After waiting several months, during which time these animals presented no clinical signs or symptoms of the disease, he injected emulsions of their various organs into normal rabbits' testicles and again obtained negative results. He interpreted these experiments as follows: Once the resistant state was fully developed, the sterilizing of the animal tissues with spirocheticides could not disturb the immunity which these animals possessed against the particular strain of *Spirochaeta pallida* used. However, when these same animals, possessing this particular strain-immunity following the chemical sterilization, after a due interval were reinoculated with a different strain of *Spirochaeta pallida*, nearly 50 per cent developed chancres at the site of inoculation. A large percentage of those apparently possessing chancre immunity proved (as indicated by positive lymph node transfers) to be infected, showing that the resistant state, as far as the rabbit is concerned anyway, is strain specific and not species specific. There is no time to give you the arguments, pro and con, which I found supporting one or the other of these two theories. It would seem, however, that as our knowledge is advancing, more and more support is being given to the Chesney theory.

Let me say here that Brown and Pearce, from the results of experiments with syphilis on rabbits, deduced two laws that regulate the biologic events taking place during experimental infection. Briefly, they are: The law of inverse proportions (the more intensive the early manifestations, the less intense are the late manifestations of the disease) and the law of sequence (by which the various systems of tissues are affected successively). This law is clearly evident in human syphilis when the integument, the internal organs, the cardiovascular system and the central nervous system are affected successively and in combination with great regularity.

THE NEISSERIAN AND CHESNEY "DOCTRINES"

Should the findings observed in animals prove to be applicable to man, it will be important for us as clinicians to know which of these two

theories is correct. If the Neisserian Doctrine is the correct one, it is obvious that our present method of treatment is proper; that is, the rapid sterilization of the tissues with spirocheticides before the invading organisms can become enmeshed in areas of fibrous isolation due to the accompanying obliterative endarteritis. We must keep in mind, however, that, in view of our recent experimental knowledge, the reinoculation test as a criterion of cure for syphilis is of no value, for, as I have already pointed out, there is no assurance that the immunity in this regard is anything more than a "chancre immunity."

If, on the other hand, the "Chesney Doctrine" is the correct one, the institution of early therapy would prevent the development of the resistant state. This would deprive the host of a protective mechanism with which to combat serious later manifestations should a few attenuated spirochetes lurking in some poorly-nourished tissue become active. Following Chesney's line of thought, late therapy, which does not preclude the possibility of complete tissue sterilization, would allow the tissues ample time to develop an immunity.

IN CONCLUSION

I had hoped to include in this review the cross-immunity existing between syphilis and certain other disease caused by spirochetes, especially yaws, and also to discuss the differences in immunity which exist between the sexes, as well as what rôle lipoids play in immunity and in the mechanism of the Wassermann reaction, etc., but I shall have to forego their discussion here because of the limited time.

490 Post Street.

THE RELATION BETWEEN THE ANTERIOR PITUITARY HORMONES ACTING ON THYROID GLAND AND ON OVARY*

By LEO LOEB, M.D.
W. C. ANDERSON, M.D.
JOHN SAXTON, M.D.
S. J. HAYWARD
AND
A. A. KIPPEN, M.D.
St. Louis, Mo.

IN addition to the well known hormones which cause growth and maturation of large follicles and luteinization processes in the ovary, we have found in the anterior pituitaries of cattle, sheep and hog, which are generally employed for the preparation of extracts of this gland, the presence of a substance which injures the follicles. This substance can be especially readily demonstrated if we use the ovary of the guinea-pig as test organ. Implantation of guinea-pig anterior pituitary causes mainly growth and maturation of the enlarged

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